

CK-MB CORRELATION STUDY BETWEEN FLUORESCENT ENZYME IMMUNOASSAY AND RAPID ELECTROPHORESIS TECHNIQUES

LT CAROLYN E. ALEXANDER

PRISCILLA THIEL

LT COL DAVID L. McCLOY

DEPT OF BIOLOGY

HOSPITAL LABORATORY

USAF ACADEMY, COLORADO 80840

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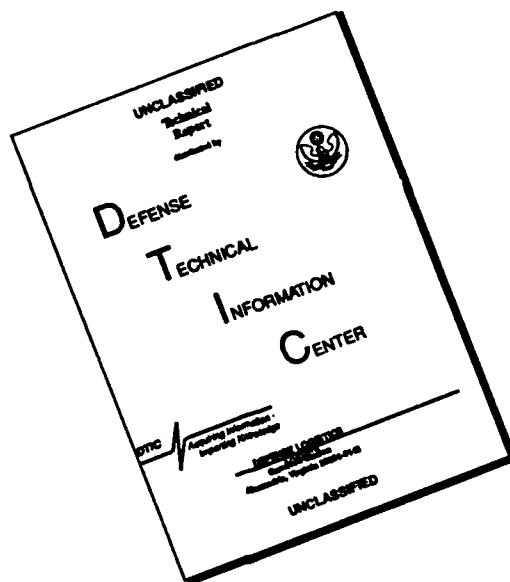
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USAF Academy, Colorado 80840

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<p>A correlation study for CK-MB analysis was performed using two state-of-the-art techniques, flourescent enzyme immunoassay on the stratus analyzer, and a rapid electrophoresis technique on the Helena REP/EDC system. Excellent diagnostic correlation between the two techniques was observed. This data suggests that the two methods can be used interchangeably for diagnostic purposes.</p>			
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CK-MB CORRELATION STUDY BETWEEN FLOURESCENT ENZYME IMMUNDASSAY
AND
RAPID ELECTROPHORESIS TECHNIQUES

BY

C1C CAROLYN E. ALEXANDER

Under The Direction Of:

Priscilla A. Thiel, MT (ASCP)
Supervisor, Clinical Chemistry

David L. McCloy, Lt Col, USAF, BSC
Chief, Clinical Laboratory Services

June 1989

Department of Biology
U.S. Air Force Academy, CO 80840

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INTRODUCTION

Creatine kinase (CK) is a dimer found primarily in skeletal muscle, cardiac muscle, and brain tissue, with small amounts found in other areas (2). There are two molecular CK subunits, designated M and B, whose combination produces three isoenzymes: CK-MM, which is primarily found in skeletal muscle; CK-MB, which is found predominantly in cardiac muscle; and CK-BB, which is found primarily in the brain.

Analysis of CK isoenzyme patterns in the sera of suspected myocardial infarction (MI) patients is one of the most accurate procedures used in the early detection of myocardial damage (5,6). An increase in CK-MB, released into the serum from myocardial cells, is usually evident within 3 to 6 hours after an infarction and generally reaches its peak concentration within 12 to 24 hours with a subsequent steady decline to normal by 72 hours (2). It is important to determine, as early as possible, if a myocardial infarction has occurred so that appropriate treatment can be administered to reduce the risk of another attack.

The two most state-of-the-art procedures available for determining the CK-MB patterns and concentrations in suspected cases of myocardial infarction are rapid electrophoresis (performed on the Helena Rapid Electrophoresis Data Center (EDC/REP), and the CK-MB Fluorescent Enzyme Immunoassay (FEIA) technique (using the DADE STRATUS Immunoassay analyzer).

The Helena rapid electrophoresis system separates the CK isoenzymes according to molecular density and overall electrical charge with the heaviest molecules remaining near the application point. The pattern is then scanned in the fluorescent spectrum using the densitometer. The clinician receives a visual graph, as well as a calculated concentration value for CK-MB present. The FEIA technique, using the STRATUS CK-MB Fluorometric Immunoassay, involves "sandwiching" the CK-MB isoenzyme between the two CK-MB antibodies to detect the concentration of CK-MB present in the sample. An index value is calculated using the CK-MB concentration as a percentage of the total CK. The clinician, using the CK-MB concentration and calculated index value, can diagnostically assess the presence or absence of a myocardial infarction occurrence.

To date, a comparative analysis has not been performed specifically between the REP and

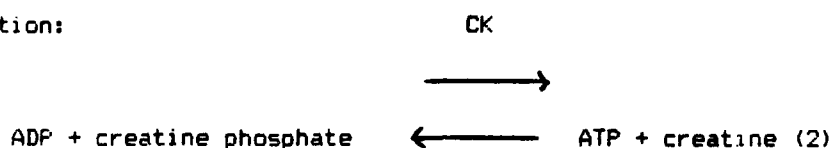
STRATUS, to determine if their results/interpretations correlate diagnostically in early detection of myocardial infarction.

The purpose of this study was to document clinical laboratory procedures used in these techniques at the USAFA Hospital and to determine if a diagnostic correlation does exist between data generated by rapid electrophoresis on the Helena REP and results obtained with the CK-MB Fluorometric Enzyme Immunoassay on the STRATUS Immunoassay system.

LITERATURE REVIEW

Creatine Kinase

Creatine kinase (CK) is an energy transfer enzyme that catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP) as shown in the following equation:



Creatine kinase, necessary for the intracellular storage and release of energy, exists as a dimer composed of two subunits, "M" and "B" (8). The combination of these subunits constitutes three isoenzymes, CK-MM, CK-MB, and CK-BB. Creatine kinase MM is found primarily in skeletal muscle, CK-MB in cardiac muscle, and CK-BB in the brain. Other tissues, such as the kidney and the diaphragm, contain significantly less activity, and the liver and erythrocytes are essentially devoid of activity (5). Table 1 provides a more detailed explanation regarding each isoenzyme's tissue and/or organ location (7).

Table 1. Location of CK Isoenzymes Within the Human Body

BB	brain, smooth muscle, thyroid, lung, prostate
MB	cardiac muscle, tongue, diaphragm, trace amount in skeletal
MM	skeletal muscle, cardiac muscle

There is also a fourth CK isoenzyme that differs from the other three both immunologically and in electrophoretic mobility. This isoenzyme, CK-Mt, located between the

outer and inner membranes of mitochondria is unaffected during myocardial infarctions and therefore would not be demonstrated (5).

Expected levels of CK activity vary according to body mass, ethnic origin, and various disease states other than a myocardial infarction. Studies have shown that the more body mass an individual has, the higher their serum CK activity (7). This is presumably the basis for both females and more slightly built individuals showing a lower serum CK activity than males and more muscular people. Ethnic origin is also important. A healthy black female generally has a higher serum activity than a white male. Lastly, some diseases will markedly increase the CK level. Duchenne's muscular dystrophy, Rocky Mountain Spotted Fever, Rhabdomyolysis, Dermatomyositis, Myoglobinuria, Polymyositis, and Reye's Syndrome are some examples (2).

The following table shows what values of total CK activity are considered expected, or normal, for each sex. These are based upon extensive studies of normal population groups conducted by Boehringer Mannheim Corporation (4).

Table 2. Expected Values of Serum CK Activity

Male:	15-130 U/L	(30°C)
	24-195 U/L	(37°C)
Female:	15-110 U/L	(30°C)
	24-170 U/L	(37°C)

Again, since expected values are affected by age, sex, diet, geographical location, and other factors, each laboratory should establish its own expected values (4). Through its own normal population study the USAFA Hospital Laboratory has verified that these expected values of CK activity recommended by Boehringer Mannheim are acceptable for this geographical location.

Rapid Electrophoresis

The Helena REP system analyzes CK isoenzyme concentrations through the use of gel electrophoresis. Gel electrophoresis is a technique in which molecules are forced across a span of gel; activated electrodes at either end of the gel provide the driving force (1). During electrophoresis the electrical current from one electrode repels the enzyme molecules while the other electrode simultaneously attracts the molecules, thus causing the isoenzymes of CK to separate on agarose gel according to their molecular weight and electrical charge (2). The macromolecules migrate away from the application point (positive electrode) toward the negative electrode when the current has been introduced.

For CK-isoenzyme analysis, electrophoresis operates by separating the creatine kinase within the serum sample into its three major isoenzymes. The electrical current causes the smallest molecular weight isoenzyme to move faster through the agarose gel, while the heavier isoenzymes remain near the application point; CK-BB is the fastest moving, CK-MM is the slowest moving, and CK-MB migrates between CK-BB and CK-MM. Figure 1 shows the relative position of the CK isoenzyme bands (2).

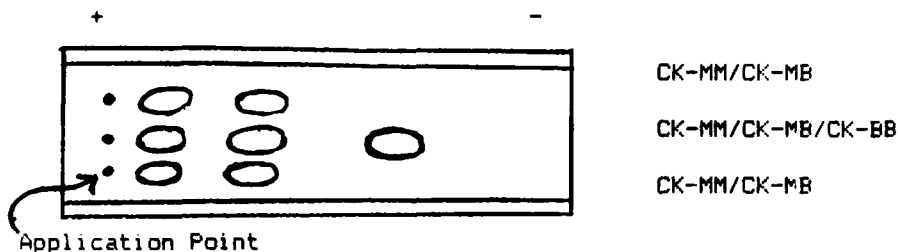
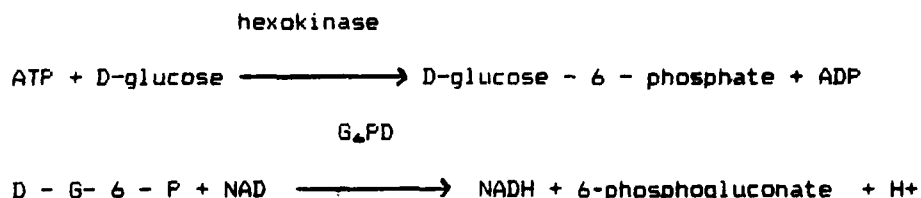


Figure 1. Positions of CK Isoenzyme Bands with the REP Electrophoresis System

The REP Isoenzyme procedure is used to analyze the qualitative and quantitative concentrations of creatine kinase by electrophoresis on agarose gel. It operates by separating the isoenzymes of CK according to their mobility, applying a glucose-based isoenzyme reagent, and then scanning the plate in the ultraviolet spectrum (i.e. 340 nm) with a densitometer which measures and analyzes optical density of the CK bands. The isoenzyme reagent utilizes the following reaction:





A qualitative or quantitative evaluation can be performed to determine the % CK-MB present. A qualitative analysis is performed by visually inspecting the plate for the presence of MB bands using an ultraviolet light. A quantitative evaluation involves using the fluorescence mode in the EDC (densitometer) to scan the electrophoretic pattern.

The gel CK plate allows the laboratory to analyze six specimens at one time. The most important consideration in the interpretation of CK isoenzyme patterns is the detection of the characteristic change of pattern over multiple examinations (i.e. the relatively fast appearance and disappearance of CK-MB).

Fluorometric Enzyme Immunoassay

Fluorometric Enzyme Immunoassay is a rapid and sensitive automated procedure for the quantitative determination of CK-MB in human serum. It is based upon the two-site sandwich immunoassay methodology. The serum sample is pipetted onto the center portion of a square piece of glass fiber paper where it reacts with a anti-CK-MB antibody. Next the sample is incubated and then conjugated with an enzyme-labeled anti-CK-MB, which reacts against a distinct site on the CK-MB molecule.

After a second incubation the labeled antibody reacts with the antibody-bound CK-MB to form a antibody/antigen/fluorescent-labeled antibody sandwich. The unbound antibody is discarded by applying a substrate wash. The substrate wash also serves to initiate the enzyme activity which can be detected by the optical system which monitors the reaction rate via fluorescence. The concentration of CK-MB present determines how rapidly the reaction rate occurs.

The STRATUS CK-MB is useful in measuring only one sample and requires approximately 20 minutes to determine the concentration of CK-MB present. An index value is calculated using

the CK-MB concentrations as a percentage of the total CK. This expediency is beneficial when a diagnosis is needed on a STAT (Emergency) basis to determine if a patient has suffered a myocardial infarction within the previous few hours.

METHODS AND MATERIALS

I. REP Isoenzyme Analysis (2)

A. Materials

1. Instrument

Helena EDC/REP System

2. - REP CK-6 Isoenzyme Plates (10)

-- contains agarose in a Tris Barbitol Buffer with 0.1% sodium azide added as a preservative

3. REP CK-6 Isoenzyme Reagent

-- includes:

Adenosine 5' - diphosphate	12mM
Creatine phosphate	90mM
Adenosine 5' - monophosphate	15mM
Magnesium Acetate	60mM
Nicotinamide adenine dinucleotide.....	6mM
N-Acetylcysteine	60mM
D-Glucose	60mM
Glucose-6-phosphate	7,500 IU/L
Hexokinase	9,000 IU/L

4. REP CK Isoenzyme Diluent (1 x 15 mL)

-- contains MES, sucrose, and 0.01% sodium azide

5. REP CK/LD Isoenzyme Control

B. Methods

1. Preparation of Isoenzyme Reagent

- reconstitute REP CK-6 Isoenzyme Reagent with 400 REP CK Isoenzyme Diluent

2. Sample Application

- a. Place 3 cups into wells 2, 3, and 4; and 3 cups into well 17, 18, and 19
(color coded with yellow strip)
- b. Add 75 uL of sample to each cup
- c. Place a blotter on sample tray
- d. Place approximately 4 mL of SURE prep (cleaning reagent) into outside washwell as well as approximately 4 mL of water in the inside washwell of the sample tray (see Figure 2)

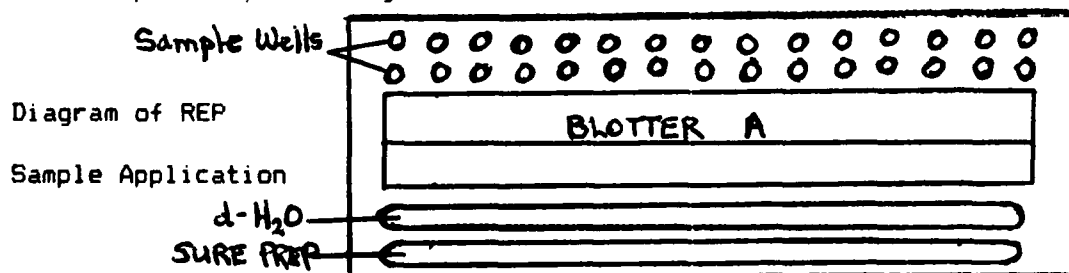


Figure 2

- e. Dispense approximately 1 mL of diluent REP Prep solution onto left side of REP chamber
- f. Remove plate from package and discard overlay
- g. Place left edge of the plate over REP chamber, aligning the round hole on the left pin. Gently lay the plate down on the REP Pre Solution, fitting the round hole over the right pin. Use a paper towel to wipe around edges of

plate, especially next to electrode posts to remove excess surfactant. Make sure no bubbles form under the plate.

- h. Place an electrode on each gel block inside the magnet posts
- i. Slide the lid into place until it snaps
- j. Place the open vial of reconstituted reagent firmly in the vial holder closest to the front of the REP (color coded with a yellow stripe)
- k. Make sure power supply button is pushed on and that the following parameters are set:

sample location (Row)	AB
sample application time	2 sec
sample absorption time	60 sec
needle wash cycles	2
needle blot time	1 sec
electrophoresis time	2 min: 30 sec
electrophoresis voltage	1100 volts
electrophoresis current	0 mA
electrophoresis temp	13°C
reagent pour time	1 sec
reagent spread cycles	4
incubation time	4 min: 30 sec
incubation temperature	45°C
dry time	4 min: 00 sec
dry temperature	54°C
standby temperature	16°C

- l. REP unit will automatically electrophorese, apply reagent, incubate and dry the agarose plate
- m. Scan the plate in the flourescent mode (EDC will automatically set for CK scans) on the Electrophoresis Data Center densitometer within one hour of drying.

II. STRATUS CK-MB Immunoassay

A. Materials

1. Instrument

DADE STRATUS Fluorometric Analyzer

2. - STRATUS CK-MB Antibody Tabs

-- a solution of mouse monoclonal anti-CK-MB IgG complexed onto the surface of glass fiber paper by the addition of goat antisera to mouse IgG

3. - STRATUS Anti-CK-MB Conjugate

-- a solution containing calf intestinal alkaline phosphatase covalently linked to mouse monoclonal anti-CK-MB Fab' in an ACES buffer, pH 7.0

- STRATUS Substrate Wash III

-- a solution containing 1 mmol/L 4-methylumbelliferyl phosphate in a diethanolamine buffer, pH 9.0

- STRATUS CK-MB Calibrators, A-F

-- solutions of human heart CK-MB in human serum base with 0.1% sodium azide added as a preservative

- STRATUS CK-MB Calibrator Diluent

-- a solution of 25 mM N-acetyl cysteine in an imidazole buffer, pH 6.7

4. Three level CK-MB Control Sera

B. Methods

1. Recalibrating the system and assaying samples in the same run:

- a. Prepare calibrators A-F and set up carousel with the calibrators run in duplicate in ascending order. If samples are to be run place them on the carousel beginning with position #13.
- b. Press CLR.
- c. In response to "ENTER TEST NUMBER 000", press 140 ENT.
- d. In response to the second "ENTER TEST NUMBER 000", press 24 ENT.
- e. In response to "NO. OF REPLICATES = 2", press ENT.
- f. In response to "STORE CONTROLS Y/N", press YES or NO.
- g. Be sure that the STRATUS Anti-CK-MB Conjugate is in the left hand reagent well.
- h. Be sure the Substrate Wash III is in the right hand reagent well.
- i. Make sure the CK-MB Tabs are in the tab loading station.
- j. In response to "REMOVE USED TABS", empty used tabs from the tab discard cage.
- k. When display reads "READY TO START? YES", start by pressing YES.

2. Running sample using stored calibration curves:

- a. Beginning with position #1, place all sample cups in the carousel with 200 uL in each sample cup.
- b. Press CLR.
- c. In response to "ENTER TEST NO. 000", press 24 ENT.
- d. In response to "STORE CONTROLS Y/N?", press YES or NO.
- e. Be sure the STRATUS CK-MB Conjugate is in the left hand reagent well.
- f. Be sure that the Substrate Wash III is in the right hand reagent wash.
- g. Make sure that the CK-MB Tabs are in the tab loading station.
- h. In response to "REMOVE USED TABS", empty used tabs from the tab discard cage.
- i. When display reads "READY TO START? YES", start by pressing YES.

III HITACHI 737 TOTAL CK METHOD

A. Materials

1. Instrument

Boehringer Mannheim Diagnostics (BMD)/HITACHI 737 Chemistry Analyzer

2. CK-NAC System Reagents (BMD)

a. Bottle 1: Buffer/Glucose

- 1) 104 mmol/L Imidazole buffer
- 2) 20.4 mmol/L D-Glucose
- 3) 10.4 mmol/L Magnesium acetate
- 4) 2.1 mmol/L EDTA

b. Bottle 1a: Enzymes/Coenzymes

- 1) 0.12 mmol ADP
- 2) 0.32 mmol AMP
- 3) 0.13 mmol NADP
- 4) 0.6 mol Bis (adenosine) pentaphosphate
- 5) 1.34 mmol NAC
- 6) > 90 U G-6-PDH
- 7) < 150 U HK

c. Bottle 2: Buffer/Glucose

- 1) 104 mmol/L Imidazole buffer
- 2) 20.8 mmol/L D-Glucose
- 3) 10.4 mmol/L Magnesium acetate
- 4) 2.1 mmol/L EDTA

d. Bottle 2a: Substrate

1) 774 mol Creatine phosphate

B. Methods

1. Reagent preparation:

a. For R1 working solution, dissolve the contents of one Bottle 1a

(Enzyme/Coenzyme) with exactly 50 mL of Bottle 1 (Buffer/Glucose).

b. For R2 working solution, dissolve 10 tablets from one Bottle 2a (Substrate)

in one Bottle 2 (Buffer/Glucose) and mix gently.

2. Analyze patient serum on Hitachi 737 for total CK results.

FINDINGS

RESULTS ON REP

Rapid Electrophoresis provides timely and accurate data regarding the percentage of CK-isoenzymes present in a serum sample. The expected percentages, determined by Helena Laboratories, for both males and females are (2):

CK-MM = 97-100%

CK-MB = 0-3%

CK-BB = 0%

The electrophoresis scan, on the Helena REP CK-6 uses a densitometer to calculate the percentage of CK isoenzymes present. After the computer analyzes the samples, a pictorial printout is constructed by the REP showing each isoenzyme fraction and the percent present. Figures 3 and 4 display actual diagrams of patients expressing normal and atypical patterns. When a myocardial infarction is suspected, an increase in % CK-MB should be observed as in Figure 4.

To be cost-effective the REP requires a minimum of 6 samples to be run simultaneously. This characteristic, combined with an average run time of 30 minutes, makes it a more preferable method of analyzing a CK-MB panel over a 24-hour period, versus using it for STAT results. Effectively interpreting electrophoretic CK isoenzyme patterns depends on the evaluation of characteristic changes on multiple examinations.

Table 4 outlines the % CK-MB results calculated using the REP. In addition, the interpretation of positive or negative MI is also included for the 45 serum samples tested. These results were used in the correlation between the REP and the results calculated using the STRATUS. (Figures 3 & 4).

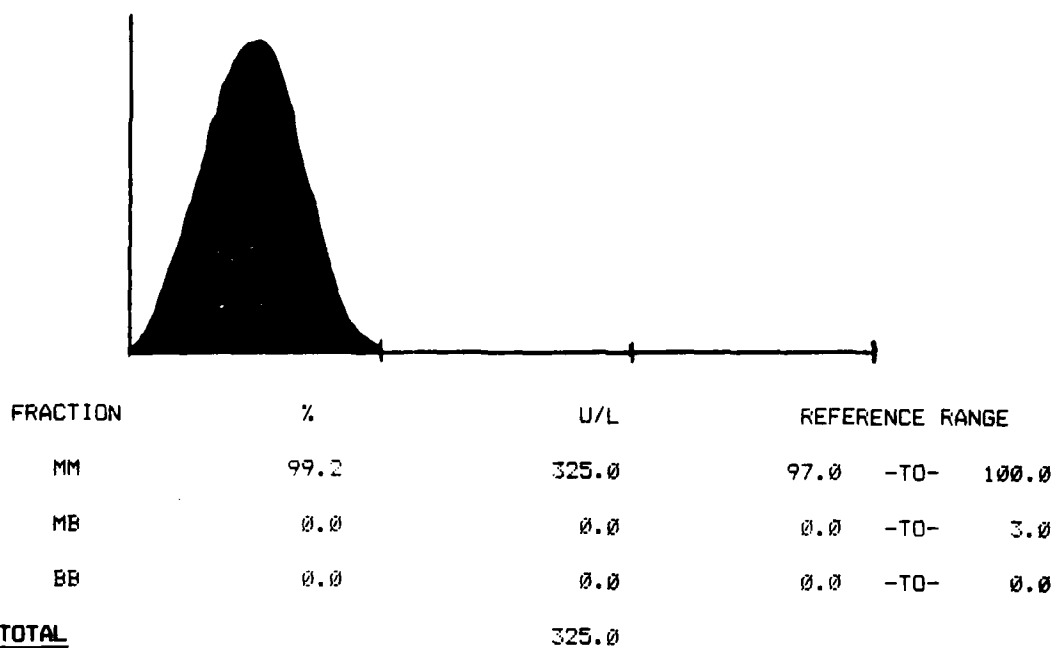


FIGURE 3. Patient showing typical isoenzyme panel with a negative myocardial infarction

Source: Patient (B), see Table 4

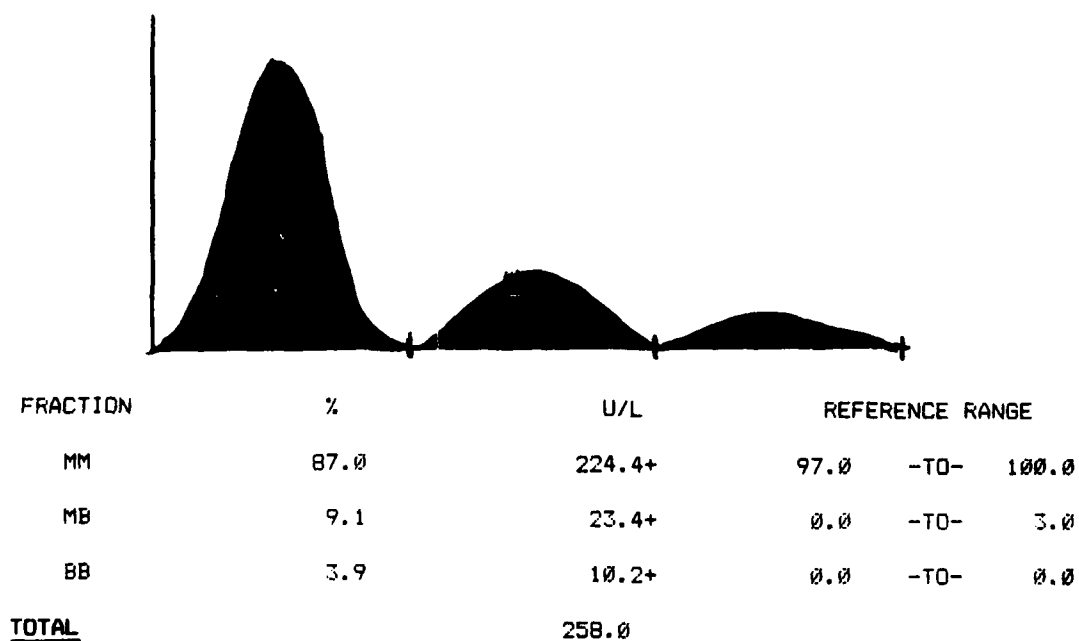


FIGURE 4. Patient showing atypical isoenzyme panel, with a positive myocardial infarction

Source: Patient (X), see Table 4

RESULTS ON STRATUS

To aid in the diagnosis of a myocardial infarction using the STRATUS CK-MB Immunoassay technique, it is necessary to calculate a CK-MB Index. Only the amount of CK-MB is determined using the STRATUS. An accurate diagnosis of a myocardial infarction requires an analysis of both CK-MB and the Total CK concentration. Figure 5 shows how CK-MB and total CK respond after a myocardial infarction has occurred. The index provides the correlation between the Total CK and the CK-MB present. The equation used to determine the index is:

$$\% \text{ Index} = \frac{\text{CK-MB ng/mL (from STRATUS)}}{\text{Total CK unit/L (from HITACHI)}} \times 100$$

After the index has been calculated, an accurate diagnosis of a myocardial infarction is possible using:

Table 3 EVALUATION CRITERIA FOR STRATUS FEIA SYSTEM

STRATUS RESULT	INDEX RESULT	INTERPRETATION
CK-MB ng/mL > 5.0	> 2.5	positive MI
CK-MB ng/mL < 5.0	< 2.5	negative MI
CK-MB ng/mL < 5.0	> 2.5	questionable MI
CK-MB ng/mL > 5.0	< 2.5	

A questionable MI means that the results lie in a gray zone where alternate testing procedures such as electrocardiograms, etc. may be necessary to determine if a myocardial infarction has occurred. (Figure 5).

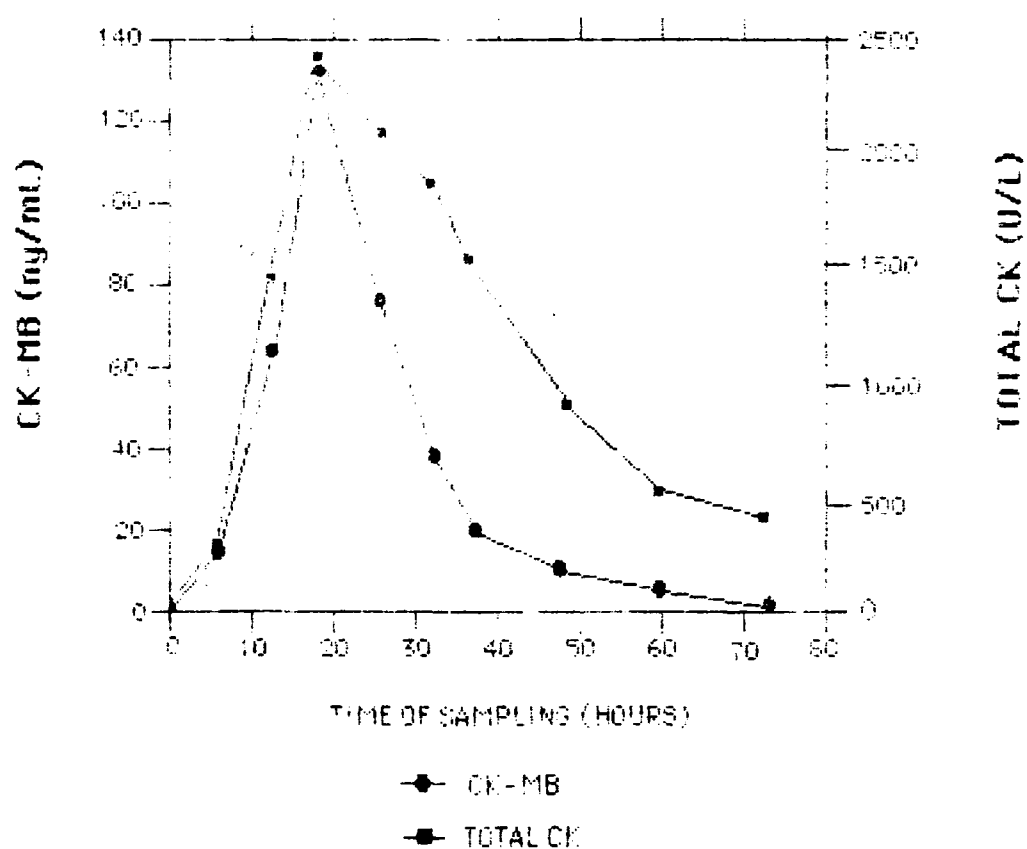


Figure 5. CK-MB and Total CK response after myocardial infarction. (3)

CORRELATION STUDIES BETWEEN REP AND STRATUS

Table 4 contains the REP results and interpretations, the Total CK, the CK-MB calculated by the STRATUS, the index, and the interpretation of a positive or negative MI occurrence using immunoassay methods. From the information provided on 45 serum samples, using both the REP and STRATUS, it was possible to evaluate the diagnostic abilities of both systems and formulate an excellent correlation between the two techniques. The correlation for diagnostic abilities with early detection of myocardial infarction between the REP and STRATUS proved to be 100%. In every case, the same diagnosis, either positive or negative for MI, was recorded for both systems. It is therefore determined that either system is accurate in its diagnostic abilities, but the applications for use of either system are more dependent upon the logistics and cost factors of the techniques themselves. Upon completing correlation studies for the MI diagnostic ability between the STRATUS and REP, a second correlation was analyzed between the actual numerical increase and decrease of the CK-MB index and the % CK-MB found using the REP. The analysis was conducted on those patients who were clinically diagnosed as positive for a myocardial infarction. Three or four serum samples were obtained from these individuals over a 24-hour interval. The correlation was based upon the change in % CK-MB and the index value over that time span.

Five patients met the requirements for this correlation study. From these 5 patients, four showed a definite pattern linking the % CK-MB to the index; that of the index following the same curve as the % CK-MB over the time span (see figures 7, 8, 9, and 10). A negative correlation was noted in one patient at 12 hours (see figure 6). It is hypothesized that this finding was the result of freezing and thawing the sample several times before it was tested. However, this hypothesis has not been confirmed.

TABLE 4
CK-MB ENZYME CORRELATION RESULTS

Patient #	Time, Date Collection	REF Result	Interpretation	Total CK	STRATUS CK-MB	Index	Interpretation
A	18 NOV 88 NO TIME	0.0% MB	NORMAL PATTERN	1203	3.5	0.7	NORMAL PATTERN
B	29 NOV 88 NO TIME	0.0% MB	NORMAL PATTERN	434	2.3	0.5	NORMAL PATTERN
B	30 NOV 88 0600	0.0% MB	NORMAL PATTERN	325	1.6	0.5	NORMAL PATTERN
E	30 NOV 88 1400	0.0% MB	NORMAL PATTERN	286	0.9	0.3	NORMAL PATTERN
E	30 NOV 88 2000	0.0% MB	NORMAL PATTERN	257	1.4	0.5	NORMAL PATTERN
C	28 JAN 89 1400	7.6% MB	POSITIVE MI	463	79.5	17.2	POSITIVE MI
C	28 JAN 89 2200	7.7% MB	POSITIVE MI	328	75.0	22.9	POSITIVE MI
C	29 JAN 89 0600	7.3% MB	POSITIVE MI	208	56.0	16.9	POSITIVE MI
D	1 FEB 89 2200	11.0% MB	POSITIVE MI	340	44.3	13.0	POSITIVE MI
D	2 FEB 89 1400	12.3% MB	POSITIVE MI	349	50.4	14.4	POSITIVE MI
E	1 FEB 89 1500	0.0% MB	NORMAL PATTERN	682	13.0	1.3	GREY ZONE, NO MI
F	2 FEB 89 1400	13.4% MB	POSITIVE MI	725	>118	16.3	POSITIVE MI
G	3 FEB 89 1400	0.0% MB	NORMAL PATTERN	259	0.0	0.0	NORMAL PATTERN
G	3 FEB 89 2200	0.0% MB	NORMAL PATTERN	282	2.6	0.2	NORMAL PATTERN
H	12 FEB 89 1730	16.7% MB	POSITIVE MI	731	>118	16.3	POSITIVE MI
H	13 FEB 89 0600	20.1% MB	POSITIVE MI	1393	>118	3.5	POSITIVE MI

CK-MB/ISOENZYMES CORRELATION RESULTS

Case	Time/Date Collection	REF Result	Interpretation	Total CK	STRATUS CK-MB	Index	Interpretation
1	14 FEB 89 0700	0.0% MB	NORMAL PATTERN	428	2.1	0.5	NORMAL PATTERN
2	14 FEB 89 1100	0.0% MB	NORMAL PATTERN	244	0.0	0.0	NORMAL PATTERN
3	14 FEB 89 2000	0.0% MB	NORMAL PATTERN	201	0.0	0.0	NORMAL PATTERN
4	14 FEB 89 2100	0.0% MB	NORMAL PATTERN	376	4.2	1.1	NORMAL PATTERN
5	25 FEB 89 1330	25.9% MB	POSITIVE MI	1858	41.8	6.3	POSITIVE MI
6	25 FEB 89 1330	9.1% MB	POSITIVE MI	1017	10.1	9.9	POSITIVE MI
7	25 FEB 89 1340	0.0% MB	NORMAL PATTERN	594	5.2	1.9	NORMAL PATTERN
8	25 FEB 89 1330	15.4% MB	POSITIVE MI	994	56.8	5.7	POSITIVE MI
9	25 FEB 89 1330	14.9% MB	POSITIVE MI	784	58.5	7.3	POSITIVE MI
10	25 FEB 89 1330	26.4% MB	POSITIVE MI	1420	41.8	11.3	POSITIVE MI
11	25 FEB 89 1330	0.0% MB	NORMAL PATTERN	656	8.4	1.3	NORMAL PATTERN
12	25 FEB 89 1200	7.3% MB	QUESTIONABLE FOR MI	203	6.2	3.2	POSITIVE MI
13	2 MAR 89 0100	0.0% MB	NORMAL PATTERN	1254	4.5	0.4	NORMAL PATTERN
14	2 MAR 89 0830	0.0% MB	NORMAL PATTERN	1274	0.6	0.063	NORMAL PATTERN
15	11 MAR 89 0600	15.7% MB	POSITIVE MI	451	34.4	0.9	POSITIVE MI
16	11 MAR 89 1400	4.3% MB	POSITIVE MI	570	15.0	5.3	POSITIVE MI

CK-ISOENZYME CORRELATION RESULTS

Patient #	Time/Date Collection	REP Result	Interpretation	Total CK	STRATUS CK-MB	Index	Interpretation
U	11 MAR 89 2200	10.6% MB	POSITIVE MI	461	26	5.6	POSITIVE MI
U	12 MAR 89 0645	7.8% MB	POSITIVE MI	388	17.7	4.6	POSITIVE MI
V	13 MAR 89 1800	0.0% MB	NORMAL PATTERN	105	0.9	0.9	NORMAL PATTERN
V	13 MAR 89 2400	19.2% MB	POSITIVE MI	3360	>119	13.5	POSITIVE MI
V	14 MAR 89 0600	18.0% MB	POSITIVE MI	2854	>119	14.2	POSITIVE MI
V	14 MAR 89 0600	0.0% MB	NORMAL PATTERN	1034	6.8	0.7	NORMAL PATTERN
X	14 MAR 89 1250	5.8% MB	POSITIVE MI	236	14.2	6.0	POSITIVE MI
X	14 MAR 89 2200	5.0% MB	POSITIVE MI	321	7.0	2.2	GRAY ZONE
X	15 MAR 89 0600	9.1% MB	POSITIVE MI	258	13.6	5.3	POSITIVE MI
Y	16 MAR 89 1630	16.8% MB	POSITIVE MI	662	70.7	10.5	POSITIVE MI
Y	16 MAR 89 2300	12.5% MB	POSITIVE MI	799	85.4	10.6	POSITIVE MI
Y	17 MAR 89 0600	15.4% MB	POSITIVE MI	628	58.5	9.3	POSITIVE MI
Y	17 MAR 89 1100	11.2% MB	POSITIVE MI	467	36.4	7.8	POSITIVE MI

FIGURE 6
Correlation Studies
on
Patient
C

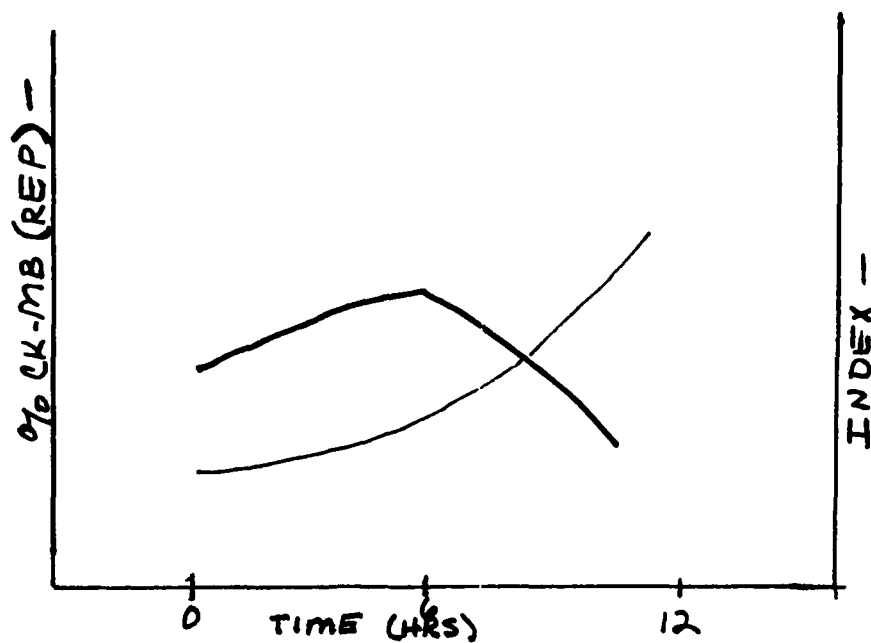


FIGURE 6

FIGURE 7
Correlation Studies
on
Patient
U

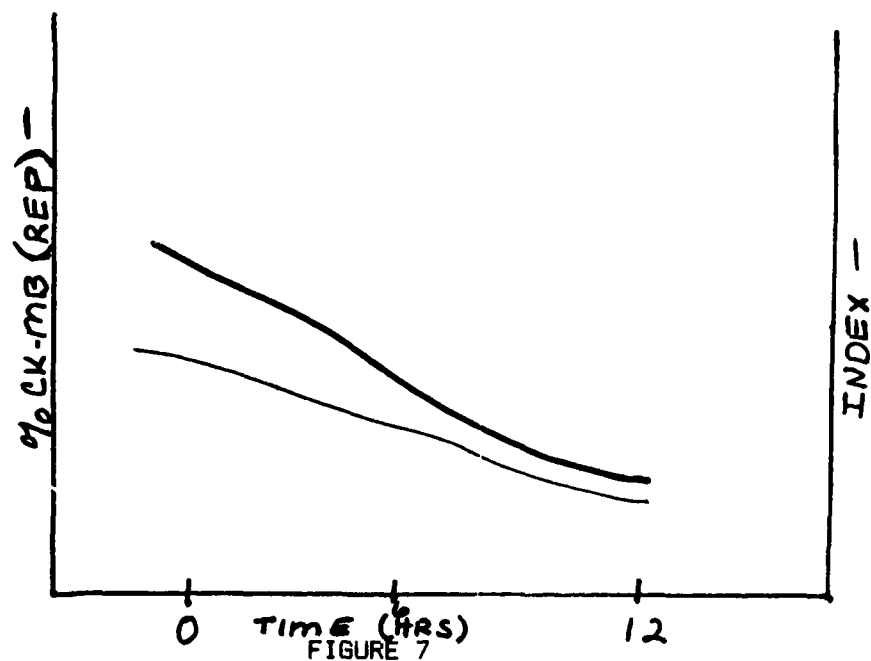


FIGURE 7

FIGURE 8
Correlation Studies
on
Patient
V

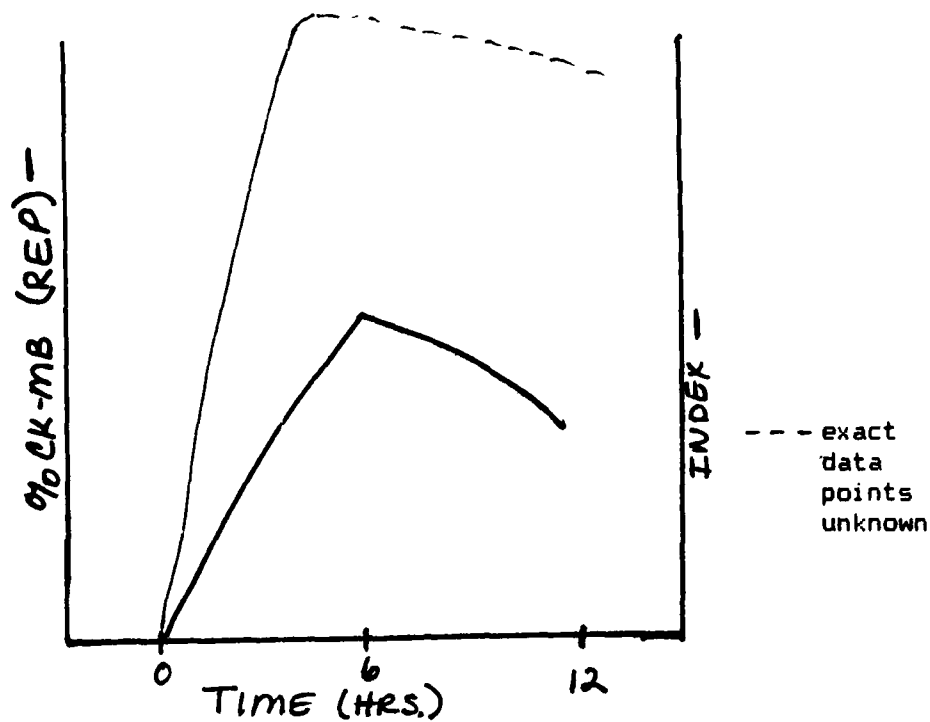


FIGURE 8

FIGURE 9
Correlation Studies
on
Patient
X

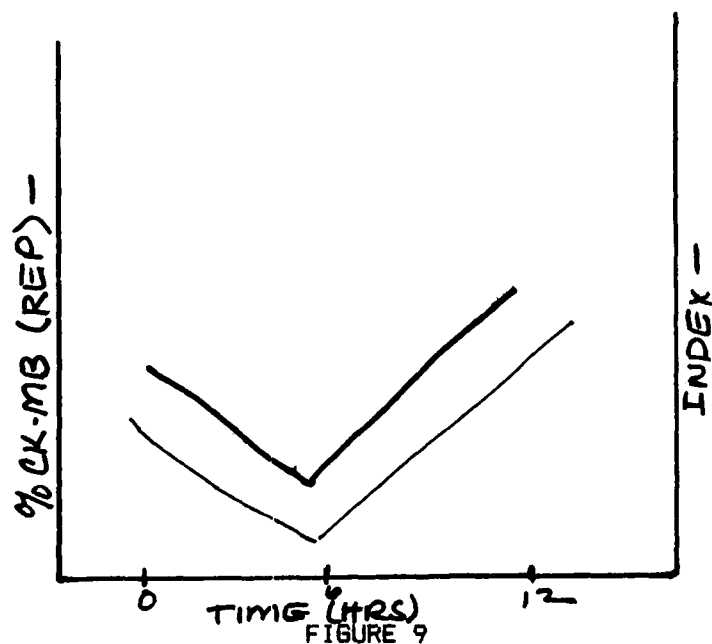


FIGURE 9

FIGURE 10
Correlation Studies
on
Patient
Y

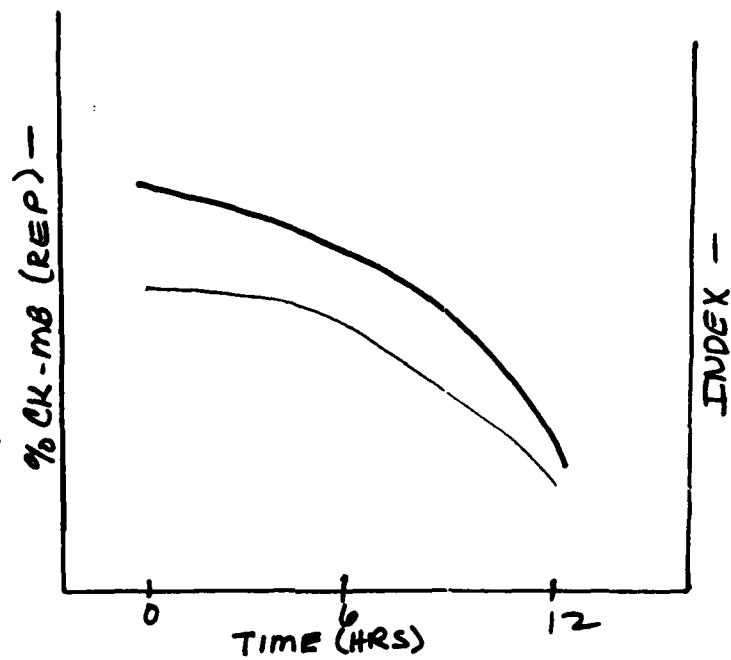


FIGURE 10

DISCUSSION AND RECOMMENDATIONS

The USAFA Clinical Chemistry laboratory, upon completion of this study, has implemented a practical and cost-effective protocol using both the REP isoenzyme technique and the STRATUS CK-MB Immunoassay procedure. Since both procedures have proven equally effective in early detection of myocardial infarction, the protocol established was primarily based on logistic as well as cost factors. Table 5 presents an overall analysis of the pros and cons of each method under discussion.

TABLE 5

PROS AND CONS OF REP VERSUS STRATUS

Rapid Electrophoresis

PROS

- visual inspection of bands possible
- gives excellent interpretation of CK isoenzyme pattern changes over multiple examinations
- excellent recovery and accuracy
- detects all CK isoenzyme dimers
- good reproducibility

CONS

- low CK-MB levels can be confused with erratic baseline problems
- requires technical skill and interpretative ability by laboratorian
- system minimum of 6 specimens per plate
- requires approximately 30 to 45 minutes for completion

STRATUS CK-MB Immunoassay

PROS

- requires little technical skill and interpretative abilities
- simplest of all the procedures to perform
- system allows for single sample analysis
- completion time of approximately 15 to 20 minutes

CONS

- macro amounts of CK-BB can interfere
 - poor precision at very low levels of CK-MB
 - no visual picture of CK-isoenzyme pattern
 - interpretation difficult in grey zone levels
-

The protocol now in effect utilizes the STRATUS CK-MB Immunoassay analysis for admission evaluation of CK-MB levels in possible myocardial infarction cases. The STRATUS provides single-specimen analysis, ease of operation and result interpretation, and gives the clinician excellent data for immediate treatment prerogatives. The USAFA Clinical Chemistry Laboratory uses the REP system to evaluate the patient's condition over the first twenty-four hours after admission. All specimens drawn in the first twenty-four hour period can be analyzed on a single six-specimen plate to give the clinician a visual and quantitative pattern of the clinical course over an extended period of time. The informal feedback received from the clinical staff indicates that this protocol is very effective, fast, and helpful in early detection of myocardial infarction.

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